13. The method according to claim 1 that in the step (3), a two-dimensional scattergram is produced from the intensity of the scattered light and the intensity of the fluorescence from the first fluorescence-labeled antibody, and the granulocytic cells obtained in step (3) are distinguished on the two-dimensional scattergram.

14. The method according to claim 1 that in the step (5), a two-dimensional scattergram is produced from the intensity of the fluorescence from the second fluorescence-labeled antibody and the intensity of the fluorescence from the third fluorescence-labeled antibody, and the neutrophilic cells are classified according to degrees of maturity on the two-dimensional scattergram.

C2 Cont

REMARKS

Rejection Under 35 U.S.C. §112, Second Paragraph

The Examiner has rejected claims 1-14 under 35 U.S.C. § 112, second paragraph, as being indefinite. Applicant addresses each asserted grounds for said rejection as follows.

With respect to Claim 1, step 1, part b, Applicants notes that examples of neutrophillic types are set forth in the specification at page 8, lines 4-7. Given the description in the specification, which provides support for the claimed features, Applicants respectfully traverse the finding that the wording is indefinite and believes no amendment is required.

With respect to Claim 1, step 3, Applicants have amended this wording to eliminate "defining a group" and instead inserts the wording "classifying" therefor. Applicant

submits this amendment addresses and resolves the particular objection raised. Support for this amendment appears in the paragraph bridging pages 11 and 12 of the specification.

With respect to Claim 1, step 4, Applicants have substantially adopted the Examiner's proposed language and believes the amendment addresses and resolves the particular objection raised. Support for this amendment appears at page 13, lines 1-15 of the specification.

With respect to Claim 1, step 5, Applicants have amended the claim to add antecedent support. Also with respect to step 5, Applicants have also adopted the Examiner's proposed language with respect to other objectionable wording in the claim. Applicant submits these amendments address and resolve the particular objection raised. Support for these amendments appears in the specification, starting at page 13, line 16 and continuing to page 14, lines 18.

With respect to Claim 2, Applicants have amended wording in the claim to conform to amended wording provided as to claim 1. No new matter has been added and support for the amendments is also that supporting analogous amendments in claim 1.

With respect to Claim 12, the wording "neutrophillic cells" has been modified to clarify the "group" as derived from the granulocytic cells obtained in step (3). This amendment mirrors that made to Claim 1, step (4). No new matter has been added.

With respect to Claim 13, similarly, the granulocytic cells are now defined as those obtained in step (3). No new matter has been added.

With respect to Claim 14, Applicants have amended the wording in the claim to reference "degrees" of maturity, according to the Examiner's suggestion and in accord with Figures 4-5 of the specification. No new matter has been added.

Applicant submits the above amendments and arguments satisfy all outstanding objections as to indefiniteness raised with respect to the Section 112, paragraph 2, rejection. No new matter has been added. Entry and approval of the amendment and withdrawal of the rejection is solicited.

Rejections Under §102(b)

Claims 1-10 and 12-14 were rejected under 35 U.S.C. §102(b) as anticipated by Bowen, *et al.*, *Laboratory Hematology* (1997) 3:292-298 ("Bowen") and as being anticipated by Loken et al., EP 0317516, ("Loken"). (Office Action, p. 6). Applicants continue to respectfully traverse these grounds for rejection and respectfully request the Examiner reconsider these findings in view of Applicants present and prior arguments.

At the outset, Applicants wish to emphasize that the present invention, as claimed, is a method for classifying and counting leukocytes. The claims that stand rejected include specific and discreet analytical steps which are not disclosed by either Bowen or Loken. Applicant submits that in issuing the rejection (of which the present Office action is only the latest formulation thereof) the Examiner has not regarded the method claimed, but continues, improperly, to provide her own assessment of the results she considers the method to yield and to find disclosure in Bowen and Loken accounting for said results. This falls well short of a demonstration of "identity of invention" required to maintain a rejection under Section 102. *Glaverbel Societe Anonyme v. Northlake Mktg. & Supply,* 33 USPQ2d 1496, 1498 (Fed. Cir. 1995). To demonstrate such identity, *each and every element* recited in a claim must be found in a single prior art reference and arranged as in the claim. *In re Marshall*, 198 USPQ 344, 346 (CCPA 1978); *Lindemann Maschinenfabrik GMBH v. American Hoist and Derrick Co.,* 221

USPQ 481, 485 (Fed. Cir 1984). This includes affirmative steps recited with respect to a method. If the Examiner cannot account for such steps, as she has not, she simply cannot maintain the rejection. As each of the analytical steps claimed have not been accounted for in Bowen or Loken (as will be further explained herein), hence, the Examiner must withdraw the rejection.

Applicants will demonstrate that the Examiner has not satisfied these requirements as to the citations. Keeping in mind the above legal principles, Applicants turn to the individual citations.

a. §102(b) Rejection based on "Bowen"

Claims 1-10, and 12-14 were rejected under 35 U.S.C. §102(b) as anticipated by Bowen, *et al.*, *Laboratory Hematology* (1997) 3:292-298 ("Bowen"). For the reasons further set forth below, this rejection, respectfully, is traversed.

As the Examiner herself summarizes Bowen, it teaches that "bone marrow was aspirated into blood collection tubes, stained using different monoclonal antibodies, then erythrocytes were lysed using Ortho Lyse." (Paper No. 13, page 5). Additionally, "[f]ive parameters which include SALS, FALS, Tri-color, FITC and PE were measured using flow cytometry." (Paper No. 13, page 5).

These are the only affirmative steps in a method that the Examiner refers to in support of the rejection. Clearly, the Examiner did not, and cannot, account for in Bowen all of the steps recited by Applicants in its claims. For instance, the affirmative step of "distinguishing eosinophils and neutrophillic cells...on the basis of intensity of the fluorescence from the first fluorescence-labeled antibody and the intensity of the fluorescence from the second or third

fluorescence-labeled antibody" is not disclosed in Bowen. Merely surmising that these steps *can* be done using antibodies disclosed in Bowen is not demonstrating these affirmative steps are in fact disclosed.

We are not concerned with what Bowen "can" say, but what it in fact discloses, and clearly the Examiners' inferential and indirect descriptions demonstrate Bowen does not disclose Applicant's specific claim limitations. Thus, the Examiners' analysis fails to properly support the Section 102 rejection. *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 9 USPQ2d 1913 (Fed. Cir. 1989) ("The identical invention must be shown in as complete detail as is contained in the ...claim").

Because each and every element of claim 1, and therefore the other claims dependent on claim 1, is not identified to be present in Bowen, the rejection fails to set forth a *prima facie* case of anticipation. Accordingly, for the reasons set forth above, withdrawal of the rejection respectfully is requested.

b. 102(b) Rejection based on "Loken"

Claims 1-10 and 12-14 were also rejected under 35 U.S.C. §102(b) as being anticipated by Loken et al., EP 0317516, ("Loken"). (Office Action, p. 6). For the reasons set forth below, this rejection, respectfully, is traversed.

In making the rejection, the Examiner contended that Loken discloses "a method and kit for classifying and counting lineages and stages of hematopoietic cells including leucocytes." (Paper 13, p. 6). After a further discussion of the Loken disclosure, the Examiner concluded: "Loken discloses that by combining intensity of light scatter (FALS or SALS) and

fluorescence intensity by different fluorochromes, various cell lineages and stages can be distinguished." (Paper 13, p. 7).

Yet, as Applicants have previously argued (and the Examiner has not addressed in the most recent Office Action) Loken does not disclose that the identification of cell lineages and stages is carried out specifically on the basis of a side scattered light and a first antibody or on the basis of intensity of the fluorescence from a second and a third antibody (using Applicants' claim parlance). Therefore, at the very least, these steps of claim 1 are not present in Loken. Moreover, use of a scattergram to classify the foregoing, as recited in Claims 13 and 14, is also not disclosed in Loken. Demonstrably, Loken does not include all the elements recited by Applicants.

Because each and every element of claim 1 is not identified by the rejection to be present in Loken, the rejection fails to set forth a prima facie case of anticipation. Accordingly, for the reasons set forth above, withdrawal of the rejection respectfully is requested.

Rejection Under §103

Claim 11 was rejected under 35 U.S.C. §103(a) as unpatentable over Bowen in view of McCarthy, *et al.*, *Journal of Immunological Methods* (1993) 163:155-160 ("McCarthy"). (Office Action, p. 8). For the reasons which follow, Applicants respectfully traverse this rejection.

Applicants note that the Examiner has not addressed Applicants' arguments with respect to this rejection save for stating "claim 11 is said to be suggested by the combination of the teaching of both Bowen and Loken with McCarthy." (Paper No. 13, page 10). Yet, apart from the erroneous characterization of the rejection (Loken is not cited as a reference under

Section 103), the Examiner's statement provides no analysis, no support and, perhaps most significantly, no citation to anything in either Bowen or Loken motivating their combination with McCarthy. Absent a demonstration of some motivation to combine, the rejection cannot stand. *In re Rouffet,* 149 F. 3d 1350; 47 USPQ2d 1453 (Fed. Cir., 1998).

Moreover, Applicants reiterate their prior arguments, which presently stand unaddressed.

The disclosure of Bowen has been discussed previously with respect to the respective rejections under §102(b).

McCarthy discloses a procedure for the quantitation by flow cytometry of function-associated antigens on neutrophils and monocytes in unlysed, unfixed, peripheral blood samples. (Abstract). McCarthy further discloses that Ficoll-Hypaque or dextran sedimentation can be used to purify peripheral blood neutrophils prior to labeling and flow cytometry. (Page 155, second column). McCarthy also discloses that such purification techniques "can by themselves induce changes in the expression of surface antigens." (Page 155, second column). McCarthy teaches that cooling blood samples to minimize metabolic changes, then labeling and analyzing the samples promptly, may minimize opportunity for activation responses and chemically induced changes. (Page 156, column 1, paragraph 2). McCarthy discloses using this technique may avoid potential "artifacts induced by the use of fixatives, erythrocyte lysing agents or leucocyte preparation techniques." (Page 159, column 2, second paragraph).

The Examiner acknowledged that Bowen does not teach a step wherein leukocytic cells are fluorescence-stained after erythrocytes are removed, as recited in Claim 11. (Office Action, p. 9).

To fill the acknowledged gap, the Examiner originally relied on McCarthy for teaching that "procedures of cellular separation or removal from other cellular populations are conventional and well-known in the art so that an issue of when such a purification or separation procedure is introduced into a method of flow cytometric analysis, i.e. before or after binding of a label to desired cells, is an obvious design choice." (Paper No. 13, p. 9).

The Examiner then contended that "it would have been obvious to one of ordinary skill in the art at the time of the instant invention to separate and purify leucocytes, such as neutrophils, from other cellular components such as erythrocytes using Ficoll-Hypaque and dextran sedimentation, as taught by McCarthy, prior to labeling of leucocytes in the cytometric analysis method of Bowen..." because McCarthy teaches that "such procedures...are conventional and well known in the art..." (Paper No. 13, p. 9)

As discussed, Bowen fails to disclose, teach, or suggest all elements of Applicants' claim 1, from which claim 11 depends. Thus, for reasons already stated, Bowen fails to account for all the elements of the base claim 1, from which claim 11 depends. Therefore, the rejection must fail, before McCarthy is even considered.

To be sure, the Examiner has only reiterated her prior rejection and has not apparently considered at all Applicant's prior response to this rejection and this combination of references. Applicants argued that McCarthy does not teach removal of erythrocytes prior to fluorescence staining (see Abstract). It discloses "purifying blood neutrophils prior to labeling" (page 155, column 2) but points out the drawbacks of this in proposing an alternative procedure. (see paragraph bridging page 155, column 2 to page 156, column 1). McCarthy, therefore, fails to account for that teaching identified as missing in Bowen; in fact, it teaches otherwise. This

renders the rejection improper. *In re Antonie*, 195 USPQ6 (CCPA 1977). (All properties and attributes must be considered by the Examiner.)

In addition, the Examiner has not addressed Applicants' assertion that the combination of references has no support, in part because McCarthy teaches away from Applicants' claim. As such, McCarthy teaches that it is not desirable to remove erythrocytes prior to staining. Moreover, McCarthy states "This procedure avoids potential artifacts that can occur due to the use of fixatives, erythrocyte lysing agents, or anticoagulants which are also divalent metal ion chelators." (Abstract). In attempting to frame "the state of the art," the Examiner inadvertently emphasizes the unobviousness of Applicants' claim. If McCarthy were, somehow, compatible and combined with Bowen, the disclosure would lead one away from Applicants' claim. No amount of downplaying McCarthy, *e.g.*, stating it is "only" cited to demonstrate that which is "conventional and well-known in the art", rehabilitates McCarthy's application to Bowen.

The disclosure in McCarthy goes directly against the assertion that Applicants' claim is merely "design choice." Applicant therefore respectfully submits that the cited references fail not only to disclose or teach each element of the Applicants' claims, but also fail to provide the requisite suggestion *to do* what the Applicants have done. For these reasons alone, the rejection of the claims is insufficient as a matter of law. *Ex parte Levengood*, 28 USPQ2d 1300, 1301-02 (BPAI 1993).

In sum, Bowen is insufficient to account for all claimed limitations in combination, let alone those which the Examiner acknowledges are missing from these references. The Examiner also fails to reference any motivation leading one of ordinary skill in the art to modify Bowen in view of McCarthy. Their combination is unsupported. Moreover,

McCarthy not only does not disclose, teach or suggest the desirability of doing what Applicants have claimed, it teaches away from what the Examiner regards as ordinary skill in the art as applied to Applicants' claim. On all counts, Applicants reiterate that the rejection is copiously without legal or factual foundation and should be withdrawn.

In view of the foregoing, favorable action on the merits, and allowance of all claims, respectfully is solicited.

Respectfully submitted,

By: _____

Charles T. J. Weigell
Reg. No. 43,398
BRYAN CAVE LLP
245 Park Avenue
New York, NY 10167
Tel. No.: (212) 692-1898
Fax No.: (212) 692-1900

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8/22/02 Date of Signature

EXHIBIT 1

"MARKED UP" AMENDMENTS TO CLAIMS PURSUANT TO RULE 1.121(c)

1. (Amended) A method for classifying and counting leukocytes comprising the steps of:

(1) adding to a hematological sample the following fluorescence-labeled antibodies labeled with fluorescent dyes which emit fluorescences distinguishable from each other;

(a) a first fluorescence-labeled antibody which binds specifically to leukocytes,

(b) a second fluorescence-labeled antibody which binds specifically to at least one kind of neutrophilic cells, and

(c) a third fluorescence-labeled antibody which binds specifically to at least one kind of immature granulocytic cells,

in order to stain the leucocytic cells in the hematological sample, and

removing erythrocytes from the hematological sample;

(2) analyzing the resulting hematological sample using a flow cytometer to measure at least one scattered light signal and three separate fluorescence signals;

(3) [defining a group of granulocytic cells] classifying granulocytic cells on the basis of intensity of the scattered light and intensity of fluorescence from the first fluorescence-labeled antibody;

(4) [distinguishing eosinophils and a group of neutrophilic cells in the defined group of granulocytic cells] distinguishing eosinophils and neutrophilic cells in the granulocytic

cells cbtained in step (3) on the basis of the intensity of the fluorescence from the first fluorescence-labeled antibody and the intensity of the fluorescence from the second or third fluorescence-labeled antibody;

(5) classifying [the defined group of the neutrophilic cells] the neutrophilic cells obtained in step (4) intro groups having different degrees of maturity into groups of neutrophilic cells different in degree of maturity on the basis of the intensity of the fluorescence from the second fluorescence-labeled antibody and the intensity of the fluorescence from the third fluorescence-labeled antibody, and

counting the number of cells in each of the groups.

2. (Amended) A method according to claim 1, wherein in step (3), a group of all leukocytic cells is defined and counted on the basis of the intensity of the scattered light and the intensity of the fluorescence from the first fluorescence-labeled antibody in addition to the [group of] granulocytic cells obtained in step (3), and in step (5), the ratio of the number of the neutrophilic cells [in each of the groups] obtained in step (4) different in degree of maturity with respect to the number of all the leukocytic cells is calculated.

12. (Amended) The method according to claim 1 that in the step (4), a two-dimensional scattergram is produced from the intensity of the fluorescence from the first fluorescence-labeled antibody and the intensity of the fluorescence from the second or third fluorescence-labeled antibody, and the eosinophils and the group of neutrophilic cells in the granulocytic cells obtained in step (3) are distinguished on the two-dimensional scattergram.

13. (Amended) The method according to claim 1 that in the step (3), a two-dimensional scattergram is produced from the intensity of the scattered light and the intensity of the fluorescence from the first fluorescence-labeled antibody, and the [group] granulocytic cells obtained in step (3) are distinguished on the two-dimensional scattergram.

14. (Amended) The method according to claim 1 that in the step (5), a two-dimensional scattergram is produced from the intensity of the fluorescence from the second fluorescence-labeled antibody and the intensity of the fluorescence from the third fluorescence-labeled antibody, and the neutrophilic cells are classified according to [the] degrees of maturity on the two-dimensional scattergram.